

Final Report
Agreement No. 00-0183S

“Evaluation of Potential Interferences for a Diazinon ELISA Test Kit”

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Scope of Work

- Phase I. Evaluate specific and non-specific interferences on diazinon ELISA of storm water runoff using surrogate or actual water samples, potentially including factors such as diazinon degradates, co-occurring pesticides in surface water runoff, humic materials, salinity, pH, etc.
- Phase II. Evaluate methods to mitigate the presence of potential interferences to improve ELISA selectivity, such as clean up or extraction procedure modifications.
- Phase III. Conduct analysis of DPR-provided storm water runoff samples for diazinon using ELISA to compare results with gas chromatographic analysis conducted at the CDFA Center for Analytical Chemistry to test the modifications developed in steps 1 and 2 of the study discussed above.

Background

CDFA has evaluated the Diazinon test kit from Strategic Diagnostics (Sullivan and Goh, 2000) and found it to have a positive bias compared to results obtained by gas chromatography. The authors of this study postulated that the bias could be caused by the presence of a cross-reacting species, diazoxon. An alternative explanation offered was that the bias was a result of undetermined matrix effects.

In another comparative study conducted in the winter of 2000, a similar high bias was found compared to a GC method run by California Fish and Game (Appendix IID). Although the data correlate well ($r = 0.974$), the slope indicated a bias by the ELISA method. Examination of this data revealed that the magnitude of the bias was not related to site of collection, date of collection or limited to a particular concentration range. GC data was routinely lower than the ELISA data, ranging from 7 to 82% lower. Spikes and blind spikes were routinely run during the ELISA analyses. Spiking levels were 0.1, 0.2 or 0.5 ppb. With few exceptions, the recoveries were all greater than 100%. The upper control limit was set at 150%. This level was exceeded in three out of 14 analyses and in two other analyses the level was 140 and 145%, and these high recovery levels were associated with the high diazinon spike level of 0.5 ppb. The remainder of the recoveries was between 110 and 130%.

CDFA would like to use the diazinon ELISA kit for routine monitoring of storm water runoff. However, the high bias is a cause for concern. The goal of this project is to identify, if possible, the source of the difference in the ELISA and GC results and to develop or suggest ways to ameliorate the difference.

Materials and Methods

Immunoassay analyses: Diazinon test kits were obtained from Strategic Diagnostics (Newark, NJ). The test kits had been purchased by CDFA and were transferred to UC Davis. Eighteen test kits were obtained in this manner. UC Davis obtained additional test kits directly from Strategic Diagnostics as needed. All kits were stored at 4 °C according to the

manufacturer's instruction. The samples were analyzed by the test kit according to the test kit insert (Appendix IIA) with modifications as indicated in the CDFA Center for Analytical Chemistry method #EM18.0 (Appendix IIB).

GC analyses: GC analyses were conducted in the CDFA Center for Analytical Chemistry using a method entitled "Diazinon in Surface Water" dated 3/30/92 (Appendix IIC).

Solid phase extraction: Samples were treated to solid phase extraction after the method of Villarosa et al. (1994). The columns were Varian-Bond ELUT cartridges, 500 mg (catalog number 12113027). They were preconditioned with 3 mL diethyl ether, 3 mL methanol, 3 mL methanol:water (60:40) and 3 mL nanopure water. Samples (10 mL) were passed through the pretreated cartridges by vacuum at a rate of approximately 1 mL/min. The cartridges were dried under vacuum for about 20 min. The adsorbed diazinon was then eluted with 2 x 1 mL of diethyl ether. The eluate was collected and evaporated under a gentle stream of nitrogen until a few drops remained. The sample was then brought to a volume of 10 mL with nanopure water and analyzed by immunoassay.

Results and Discussion

I. Cross reactivity

The table below lists the cross reactivity for structurally related compounds. If a complete inhibition curve was obtained the IC_{50} was calculated and a % cross reactivity determined. In other cases there was a trend toward inhibition and the % inhibition at the highest concentration tested (i.e. 5000 ng/mL) is reported. All other compounds showed no inhibition at 5000 ng/mL.

A. Structurally related compounds. The oxon form of diazinon cross reacts to a small extent (2%), but the 2-isopropyl-6-methyl-4-pyrimidinolpyrimidone leaving group cross-reaction is extremely small, inhibiting the assay only 36% at 5000 ng/mL. Both of these findings are in agreement with the paper by Beasley et al., that describes the production of the antibody and initial assay development. Although the USEPA SAP states that the 2-isopropyl-4-methyl-6-hydroxypyrimidine is more mobile and more persistent than diazinon, it is not likely the cause of the bias in ELISA results since it does not cross-react. The diazoxon is known to cross react, however recent monitoring studies by the CDFA (Domagalski, 1996) indicate that the diazoxon accounts for only 1-3% of the total amount of the diazinon load, thus also would not account for the total bias.

Table 1. Cross reactivity of structurally related compounds.

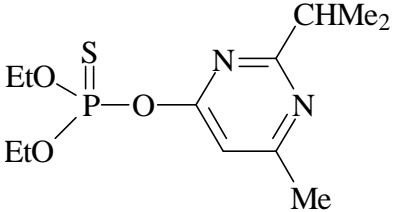
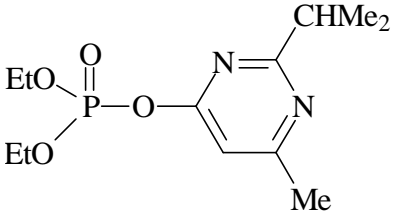
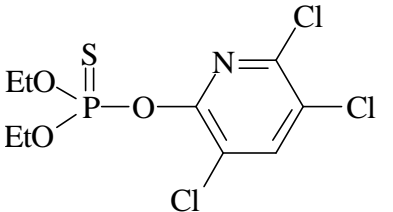
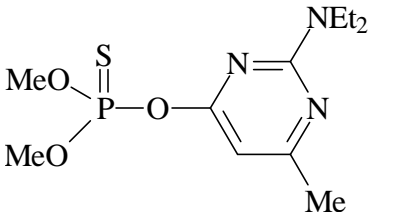
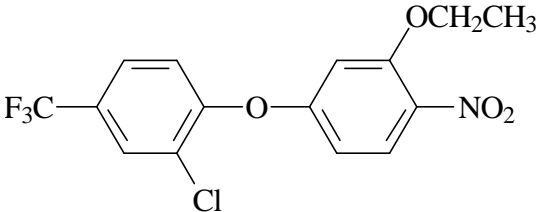
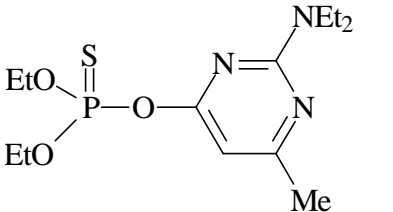
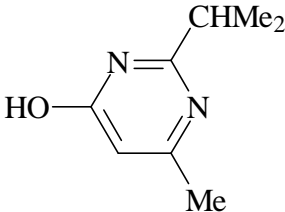
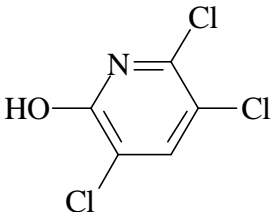
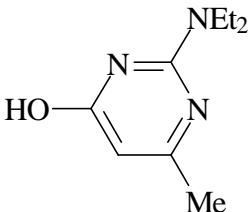
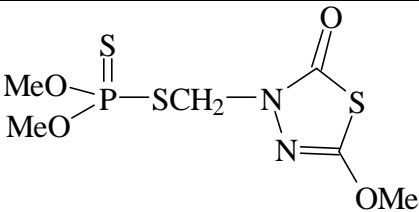
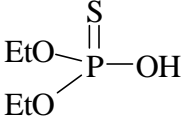
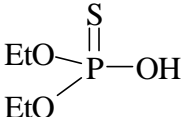
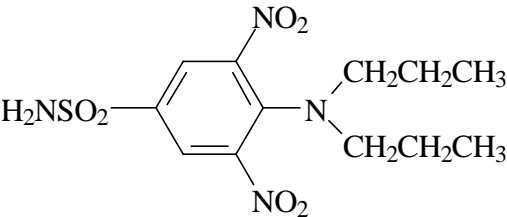
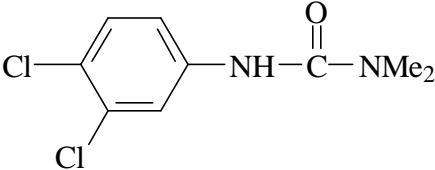
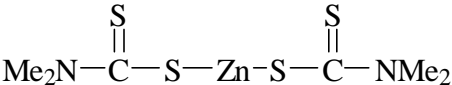
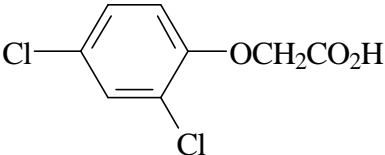
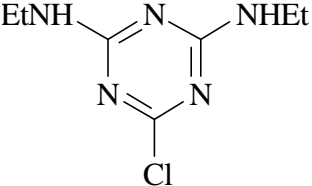
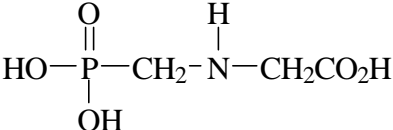
Name	Structure	IC ₅₀ (ppb)	% Cross Reactivity	% Inhibition at 5000 ng/mL
Diazinon		0.2	100%	
Diazoxon		9	2	
Chlorpyrifos		610	0.03	
Pyrimifos methyl				74
Oxyfluorfen				57
Pyrimiphos ethyl				60

Table 1. Cross reactivity of structurally related compounds (con't)

Name	Structure	IC ₅₀ (ppb)	% Cross Reactivity	% Inhibition at 5000 ng/mL
2-Isopropyl-6-methyl-4-pyrimidinol				36
3,5,6-Trichloropyridinol				37
2-Diethylamino-6-methyl-4-pyrimidinol				0
Methidathion				0
Diethyl phosphate				0
Diethylthiophosphate				0

B. High use compounds. The following table contains compounds used abundantly or during the dormant spray season. Diazinon, methidathion and oxyfluorfen are among the top ten, and are listed in the table above. Methyl bromide and 1,3-dichloropropene are also among the top ten, and were not tested. Due to their volatility, they are unlikely to be present in samples. Simazine has been found in runoff (Domalgalski, 1996).

Table 2. Cross reactivity of some "high use" compounds.

Name	Structure	IC ₅₀ (ppb)	% Cross Reactivity	% Inhibition at 5000 ng/mL
Oryzalin				0
Diuron				0
Ziram				22
2,4-D				0
Simazine				40
Glyphosate				0

C. Other pesticides tested are in the table below. Molinate and its degradates have also been found in runoff, as has carbofuran which was not tested. (Domagalski, 1996).

Table 3. Cross reactivity of some other pesticides.

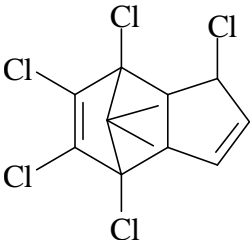
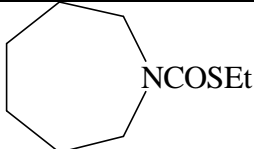
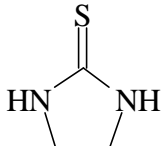
Name	Structure	IC ₅₀ (ppb)	% Cross Reactivity	% Inhibition at 5000 ng/mL
Atrazine	$\text{EtNH} \begin{array}{c} \diagup \text{N} \diagdown \\ \diagdown \text{N} \diagup \\ \text{N} \end{array} \text{NHPr} \\ \text{Cl}$			57
Thiobencarb	$\text{Et}_2\text{NC(=O)SCH}_2\text{---} \langle \text{C}_6\text{H}_4 \rangle \text{---Cl}$			42
Carbaryl	$\text{MeNHCO} \begin{array}{c} \text{O} \\ \parallel \\ \text{C} \end{array} \begin{array}{c} \diagup \\ \diagdown \end{array} \langle \text{C}_{10}\text{H}_7 \rangle$			0
Propoxur	$\text{MeNHCO} \begin{array}{c} \text{O} \\ \parallel \\ \text{C} \end{array} \begin{array}{c} \diagup \\ \diagdown \end{array} \langle \text{C}_6\text{H}_4 \rangle \text{OCHMe}_2$			0
Aldicarb	$\text{MeNHCO} \begin{array}{c} \text{O} \\ \parallel \\ \text{C} \end{array} \text{---N=CH---} \begin{array}{c} \text{Me} \\ \\ \text{C} \text{---SMe} \\ \\ \text{Me} \end{array}$			0
Trichlopyr	$\text{Cl} \begin{array}{c} \diagup \text{N} \diagdown \\ \diagdown \text{C} \diagup \\ \text{Cl} \end{array} \text{OCH}_2\text{COOH} \\ \text{Cl}$			0
Paraquat dichloride	$\text{Me---N}^+ \langle \text{C}_6\text{H}_4 \rangle \text{---} \langle \text{C}_6\text{H}_4 \rangle \text{N}^+ \text{---Me} \\ 2\text{Cl}^-$			0
Heptachlor				0

Table 3. Cross reactivity of some other pesticides (con't)

Name	Structure	IC ₅₀ (ppb)	% Cross Reactivity	% Inhibition at 5000 ng/mL
Molinate				0
Ethylenethiourea				0

D. Other potential interferents. An examination of the Pesticide Use Report shows that many inorganics are used during the dormant spray season in amounts much larger than the pesticides. Among them are copper sulfate, sulfur and mineral oil. We tested solutions of these and found the following: A 1 mM copper sulfate solution corresponded to 49 ppt of diazinon. Nanopure water saturated with sulfur corresponded to 151 ppt of diazinon. Nanopure water saturated with mineral oil corresponded to 22 ppt of diazinon.

There appear to be several organic and inorganic chemicals that can affect the assay either selectively (a cross-reactant) or non-selectively (interferent). No single compound can account for the high bias seen in the 2000 samples discussed above. However, it is possible that a combination of chemicals could be responsible for some of the bias.

II. Methods Evaluation

A. Old vs new test kits. Some of the test kits received from CDFA were near the expiration date. Tests were conducted with these “nearly” expired and new kits to determine the usefulness of the old kits for this study.

Table 4. Evaluation of old vs new test kits

Sample #	ELISA	ELISA	GC
	Old Test Kit Diazinon Found, ppt	New Test Kit Diazinon Found, ppt	
48	35	38	50
66	13	29	50
103	28	73	85
138	16	59	67
153	11	34	42

Samples were from study 199 and were measured without pretreatment.
Samples run in three well replicates on the same day.

The data in Table 4 show that the old test kits gave diazinon concentrations for these samples that were lower than might be expected compared to GC values and different from the values for the new kits. The new kits, however, gave values closer to the GC values. Most of the test kit parameters for the old kit were within the quality control values reported in the test kit insert. New kits were used where quantitative data was needed. The old kits were used in range-finding and methods development studies and in the determination of cross reactivity.

B. Recovery studies. Background water was collected from Wadsworth (Butte County) and Karnak (Sutter Bypass) collection sites. The Karnak sample contained some suspended sediment. An aliquot of each of the water types was spiked with the indicated amount of diazinon and analyzed without pretreatment. Three well replicates of each sample were run on the same day.

Table 5. Spike recovery.

Sampling Site	Nominal Spike Level ppt	Diazinon Found ppt	% Recovery
Wadsworth			
	30	38.9	129.7
	60	54.8	91.3
	100	136.4	136.4
	200	200.2	100.1
	500	478	95.6
	1000	841.7***	84.2
Karnak			
	30	31.2	104.0
	60	43.2	72.0
	100	64.2	64.2
	500	485.9	97.2
	1000	831.4***	83.1

***Indicates that absorbances were outside the range of the highest standard, 500 ppt.

The data in Table 5 indicate that recovery of diazinon from these water samples was variable with no particular relationship to spike concentration. In addition, some of the spikes were outside the normally acceptable range of 80-120% recoveries. Thus, some matrix effect is presumed. To test this idea, samples were subjected to solid phase extraction.

C. Recovery studies following SPE. Background water was collected from Wadsworth (Butte County) and Karnak (Sutter Bypass) collection sites. The Karnak sample contained some suspended sediment. Aliquots (10 mL) of each of the water types was spiked with the indicated amount of diazinon in duplicate and analyzed by SPE. Three well replicates of each sample were run on the same day.

Table 6. Spike recovery following SPE

	Nominal Spike Level ppt	Mean* Diazinon Found ppt	Mean % Recovery
Wadsworth			
	60	105.0	175.0
	100	116.8	116.8
	500	485.8	97.2
Karnak			
	60	77.2	128.7
	100	105.5	105.5
	500	428.2	85.6

*n=2.

The data in Table 6 indicate that the solid phase extraction method used, results in good recovery of diazinon. From the limited concentrations tested, the SPE does seem to eliminate some interference. However, the recoveries for the 60 ppt samples are outside the acceptable range. This was not the case when the samples were analyzed directly, which implies that something may be co-eluting with diazinon that is interfering. Further work on optimizing a solid phase extraction method is needed.

D. Day-to-day variation. Data were compiled from those experiments in which the same samples were run on more than one day to examine day-to-day variation. On each day, three independent replicates of each sample were run. One set of samples was run without pretreatment. A second set of data is for samples that had been analyzed following solid phase extraction. With this limited data set, there were no differences in values obtained from day to day.

Table 7. Day-to-day variation.

Sample #	03/08/01 ELISA	03/09/01 ELISA
418	406 ± 17	408 ± 75
441	59 ± 20	56 ± 12
466	578 ± 49	493 ± 37
491	69 ± 13	81 ± 25
376	209 ± 19	222 ± 24
389	370 ± 11	217 ± 31
	02/22/01 SPE ELISA	02/23/01 SPE ELISA
376A	147 ± 21	158 ± 25
376B	129 ± 16	105 ± 7
389A	243 ± 9	253 ± 13
389B	232 ± 4	221 ± 38

Table 7 shows that samples analyzed on two consecutive days give values that are identical or overlapping within the standard deviation of the determinations.

E. Evaluation of calibration curve. The absorbances for each calibrator are used to back calculate each concentration as another estimate of the fit of the calibration curve. Table 8 is a compilation of this data collected over several months.

Table 8. "Recovered" diazinon using absorbances from calibration standards.

Nominal Diazinon Conc ppt	Mean Found Conc ppt	SD	Mean %Difference	SD
30	29.91	2.37	6.13	4.69
60	55.94	6.20	10.50	6.15
100	107.35	12.91	11.78	8.71
200	202.73	21.74	8.14	7.01
500	501.43	49.06	7.57	5.91

The curve fit data (Table 8) show that the calibrators selected fit the semi-log regression well (mean % differences between found and nominal were between 6 and 12%) and accurate quantitation should be expected within the range of calibrators used. The %CVs on the found concentrations were between 8 and 12%.

III. Sample Analysis

A. Study #199. A series of samples were collected from Wadsworth Canal over a three-month period. These samples were analyzed by GC and by immunoassay. For immunoassay the samples were analyzed without pretreatment and in addition a portion were analyzed following solid phase extraction. The MDL for the immunoassay method was set, conservatively, at 30 ppt as that was the lowest concentration tested in recovery studies. The LLD for the kit assay was 22 ppt according to the manufacturer.

Table 9. Immunoassay and GC results for samples collected for Study #199.

Sample #	ELISA Result (ppt) - direct	Std Dev	%CV	n	ELISA Result (ppt) - SPE	Std Dev	%CV	n	GC Result (ppt)
47	NT*				NT				59
48	39.8				NT				
65	NT				NT				
66	ND				NT				53
103	73.2				NT				85
138	59.6				NT				67
153	35.3				NT				42
182	ND				69.1	0.8	1.2	2	40
201	248.6	38.5	15.5	3	178.7	9.3	5.2	2	185
230	53.9				73.1	5.6	7.7	2	69
249	43.9				51.6	1.8	3.5	2	49
278	ND				42.3	2.7	6.4	2	ND
297	43.5	7.3	16.8	2	51.2	0.8	1.6	2	ND
328	609.2	63.5	10.4	4	495.5	54.9	11.1	8	536
341	218.4	30.8	14.1	4	147.6	23.5	15.9	4	164
376	200.4	8	4.0	3	132.3	17.3	13.1	6	119
389	296.3	40.5	13.7	3	215	15.9	7.4	6	154
418	362	39.8	11.0	4	292.3	73.5	25.2	3	289
441	58.5	1.3	2.2	2	50.8	2.8	5.5	2	49
466	452.6	87.8	19.4	4	282.7	51.6	18.3	6	393
491	74.7	7.8	10.4	2	50.3	12.9	25.7	2	64
555	ND			2	ND			4	ND
590	53.9				63.4	23.8	37.5	2	ND

*NT = not tested; ND = not detectable. Immunoassay MDL = 30 ppt; GC MDL = 40 ppt.

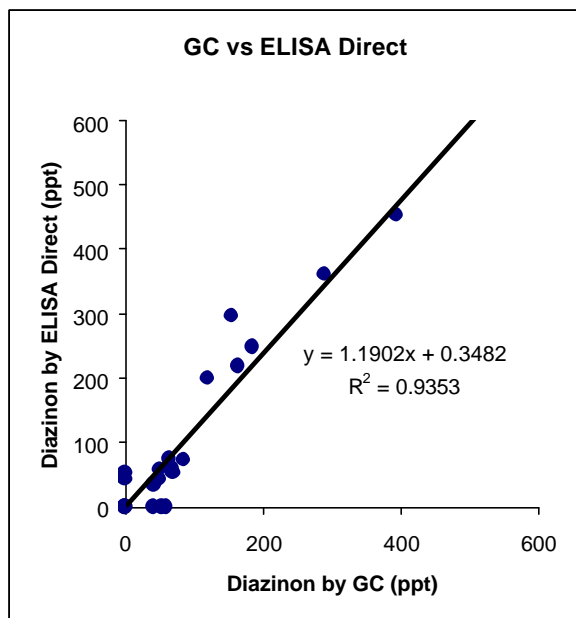


Figure 1. Comparison of analysis of samples by GC and by immunoassay without sample pretreatment.

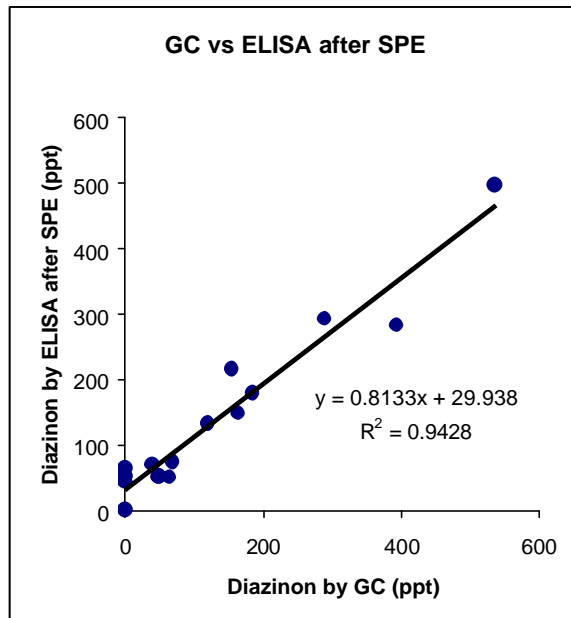


Figure 2. Comparison of analysis of samples by GC and by immunoassay following SPE.

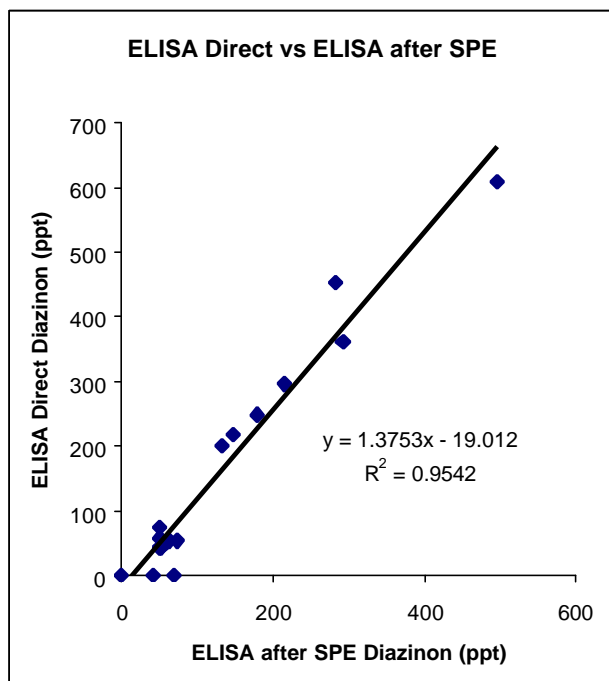


Figure 3. Comparison of analysis of samples by immunoassay without pretreatment (direct) and by immunoassay following SPE.

Figure 1 shows that the analysis of samples directly by ELISA agrees well with the GC data, with a slight high bias for the immunoassay. After solid phase extraction, values between immunoassay and GC still agree well, but the bias for the immunoassay is low. An examination of Table 9 shows that the high bias for samples analyzed by immunoassay and run directly is primarily due to samples containing high levels of diazinon (>200 ppt by immunoassay). Following cleanup, the data by immunoassay for these high level samples agrees better with GC data, implying that if a matrix effect was present, it was eliminated. However, because the values are low, there may be a recovery problem. This supports the idea that further work is necessary on the solid phase extraction method.

B. Study #201. A series of samples were collected during rain events in February 2001. The samples were analyzed by both GC and ELISA and the results shown in Table 10.

Table 10. Diazinon concentrations in water samples by ELISA and GC, organized by sample number.

Site	Date/Time	Sample #	ELISA Result (ppt)	GC Result (ppt)
Sacramento Outfall	2/10/01 10:55	10	ND*	125
Alamar	2/10/01 10:50	12	ND	ND
Butte Slough @ Lower Pass Road	2/9/01 12:00	60	ND	30
Mud Creek	2/9/01 16:00	87	ND	48
Lindo Channel	2/9/01 16:55	89	83	129
Big Chico Creek @ River Road	2/9/01 17:55	91	95	62
Big Chico Creek @ Rose Avenue	2/9/01 19:10	94	ND	ND
Big Chico Creek @ River Road	2/10/01 8:50	96	ND	ND
Big Chico Creek @ River Road	2/10/01 10:20	121	ND	ND
Stony Creek	2/10/01 11:20	122	ND	ND
Sacramento River @ Hamilton City	2/10/01 12:10	126	ND	ND
Mud Creek	2/10/01 13:10	130	ND	20
Lindo Channel	2/10/01 13:40	132	155	185
Sacramento River @ Hamilton City	2/11/01 11:50	133	ND	ND
Sacramento Outfall	2/11/01 11:30	158	73	141
Obanion South	2/11/01 12:03	160	87	143
Obanion North	2/11/01 12:17	162	ND	38
Sacramento Outfall	2/12/01 12:00	164	112	107
		168	ND	61
Sacramento River @ Hamilton City	2/14/01 11:10	182	ND	ND
Little Chico Creek	2/10/01 14:50	218	ND	20
Big Chico Creek @ Rose Avenue	2/10/01 15:25	221	ND	ND
Big Chico Creek @ River Road	2/10/01 16:00	223	ND	22
Big Chico Creek @ Rose Avenue	2/11/01 8:55	225	ND	20
Big Chico Creek @ River Road	2/22/01 9:45	227	ND	ND
Big Chico Creek @ River Road	2/9/01 9:20	242	ND	ND
Stony Creek	2/9/01 10:55	244	ND	21
Big Chico Creek @ Rose Avenue	2/9/01 12:10	247	ND	24

Table 10. Diazinon concentrations in water samples by ELISA and GC, organized by sample number (con't).

Site	Date/Time	Sample #	ELISA	GC	
			Result (ppt)	Result (ppt)	Result (ppt)
Little Chico Creek	2/9/01 13:35		249	62	133
Sacramento River @ Hamilton City	2/9/01 15:25		251	ND	ND
Sacramento River @ Colusa	2/14/01 10:00		351	ND	ND
Butte Slough @ Lower Pass Road	2/14/01 11:15		353	ND	25
Feather River @ Yuba City	2/14/01 11:50		356	ND	20
Jack Slough @ Doc Adams Road	2/14/01 12:20		358	ND	53
Sacramento Outfall	2/9/01 12:10		384	57	111
Sacramento River @ Colusa	2/10/01 10:55		434	ND	38
Butte Slough @ Lower Pass Road	2/11/01 11:25		436	ND	38
Feather River @ Yuba City	2/11/01 12:10		438	ND	ND
Jack Slough @ Doc Adams Road	2/11/01 12:45		440	ND	102
Sacramento River @ Colusa	2/12/01 10:45		442	ND	20
Butte Slough @ Lower Pass Road	2/12/01 11:15		444	ND	27
Wadsworth	2/11/01 13:00		446	322	770
Butte	2/11/01 14:05		448	71	24
Main Canal	2/11/01 14:30		450	253	175
Main Canal	2/11/01 18:00		453	55	20
Main Canal	2/11/01 18:15		455	74	134
Main Canal	2/9/01 18:30		457	50	23
Wadsworth	2/9/01 19:20		459	76	61
Butte Canal	2/9/01 22:17		461	ND	28
Main Canal	2/9/01 22:40		463	128	101
Wadsworth	2/9/01 23:20		465	192	358
Butte Creek	2/10/01 2:20		467	76	26
Butte Creek	2/10/01 10:30		470	51	24
Main Canal	2/10/01 10:55		472	77	51
Wadsworth	2/10/01 14:10		474	323	513
Butte Creek	2/10/01 15:25		476	53	23
Main Canal	2/10/01 15:40		478	61	47
Butte Creek	2/11/01 2:10		480	69	25
Feather River @ Star Bend	2/14/01 10:40		486	34	ND
Feather River @ Star Bend	2/10/01 13:00		495	42	26
Bear River @ Berry Road	2/11/01 10:30		497	ND	ND
Feather River @ Star Bend	2/11/01 13:00		500	ND	ND
Bear River @ Berry Road	2/12/01 11:00		502	27	43
Feather River @ Star Bend	2/12/01 13:00		504	ND	ND
Jack Slough @ Doc Adams Road	2/9/01 13:15		506	57	96
Sacramento River @ Colusa	2/10/01 10:20		508	ND	ND
Butte Slough @ Lower Pass Road	2/10/01 11:20		510	ND	36
Feather River @ Yuba City	2/10/01 12:10		513	ND	ND
Jack Slough @ Doc Adams Road	2/10/01 12:45		515	54	86

Table 10. Diazinon concentrations in water samples by ELISA and GC, organized by sample number (con't).

Site	Date/Time	Sample #	ELISA	GC	
			Result (ppt)	Result (ppt)	Result (ppt)
Obanion South	2/10/01 12:22		558	65	58
Obanion North	2/10/01 12:39		561	ND	25
Bear River @ Berry Road	2/9/01 10:50		584	ND	50
Bear River @ Berry Road	2/10/01 10:30		586	ND	42
Feather River @ Yuba City	2/12/01 12:30		615	ND	ND
Jack Slough @ Doc Adams Road	2/12/01 13:00		617	ND	66
Butte Creek	2/9/01 12:25		626	ND	32
Main Canal	2/9/01 12:50		630	ND	20
Wadsworth	2/9/01 16:05		632	ND	38
Butte Creek	2/9/01 18:15		635	ND	28
Main Canal	2/10/01 2:38		637	68	83
Wadsworth	2/10/01 3:20		639	160	207
Butte Creek	2/10/01 6:15		642	37	28
Wadsworth	2/10/01 7:35		648	108	1380
Butte Creek	2/11/01 6:10		661	ND	20
Main Canal	2/11/01 7:00		665	56	91
Wadsworth	2/11/01 8:25		668	294	418
Butte Creek	2/11/01 10:15		670	ND	23
Main Canal	2/11/01 10:30		672	72	95
Butte Canal	2/12/01 18:15		696	ND	ND
Obanion South	2/12/01 12:50		699	ND	31
Obanion South	2/9/01 12:50		701	ND	ND
Obanion North	2/9/01 13:10		705	ND	24
Butte	2/13/01 10:31		710	ND	21
Main Canal	2/13/01 10:45		713	ND	47
Main Canal	2/11/01 2:20		722	37	92
Wadsworth	2/11/01 3:00		724	257	453
Wadsworth	2/11/01 19:00		725	159	829
Butte Creek	2/9/01 18:55		746	ND	22
Main Canal	2/10/01 19:10		748	ND	44
Wadsworth	2/10/01 20:00		750	178	872
Butte Creek	2/10/01 22:05		752	ND	22
Main Canal	2/10/01 22:20		754	33	54
Wadsworth	2/10/01 23:00		756	212	630

* ND = not detected, below the reporting limit. The reporting limit for GC was 20 ppt. The reporting limit for the ELISA was 30 ppt.

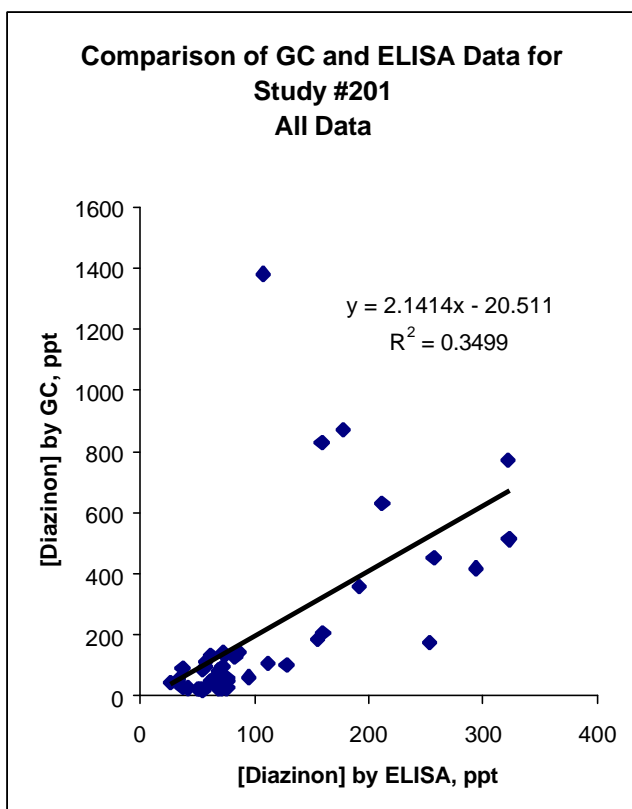


Figure 4. Comparison of GC and ELISA data for samples from study #201. All data are shown on this graph except for non-detects.

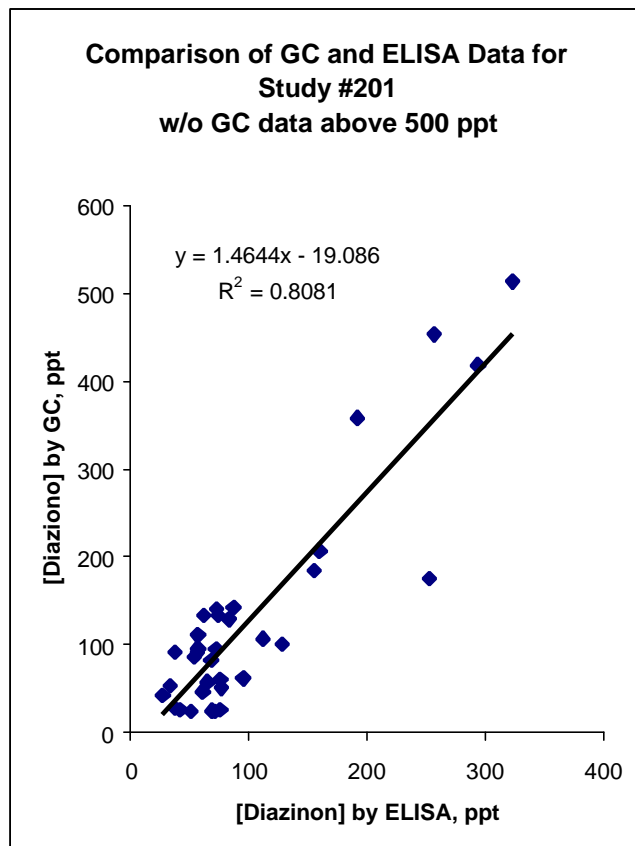


Figure 5. Comparison of GC and ELISA data for samples from Study #201. All points that were non-detects or correlated to GC values above 500 ppt were omitted.

The correlation in Figure 4 for all data points is poor. GC values were much higher than ELISA values in this study. Examination of data in Table 10 show that discrepancies were greatest at concentrations of 500 ppt and greater as measured by GC. When these data are omitted, (Figure 5), the correlation greatly improves. Nevertheless, there is still a strong high bias for GC values. This is in contrast to results of Study #199 where the high bias was in favor of the ELISA.

Conclusion

The Strategic Diagnostics test kits performed to the specifications given in the test kit insert. The curve fit data (Table 8) show that the calibrators selected fit the semi-log regression well (mean % differences between found and nominal were between 6 and 12%) and accurate quantitation should be expected within the range of calibrators used. The %CVs on the found concentrations were between 8 and 12%. The kits should be used before their expiration date to assure quality data.

The high bias detected in earlier studies was not reproduced in the work conducted here. The test kit itself, does not seem to be a problem, thus the most likely explanation is interference from the matrix and that this interferent(s) was not present this season.

The solid phase extraction method used was apparently successful in removing interferences, but further work is needed to optimize and characterize the method.

A wide variety of compounds can interfere with the assay (as seen in the cross reactivity studies), but no one compound is likely responsible for the bias seen earlier. Significant interference might be seen if several of these compounds were present simultaneously.

The correlation among study #201 immunoassay and GC data was less strong than for study #199. This might be because study #199 samples were all collected at the same sight, although sorting data in study #201 by site does not show any particular correlation to variability.

References

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